jnm/ RADIOCHEMISTRY AND RADIOPHARMACY

EFFECT OF LARGE DOSES OF ^{131}I-19-IODOCHOLESTEROL ON METAPYRALONE-INDUCED ADRENAL CORTICAL HYPERPLASIA IN DOGS

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The potential use of ^{131}I-19-iodocholesterol to treat ACTH excess Cushing's disease was evaluated in the dog. Three normal female dogs were given LD_{50} radiation doses of ^{131}I-19-iodocholesterol without producing gross or histopathologically demonstrable change of the adrenals at autopsy 3 months later. The adrenal cortices of three dogs were made hyperplastic (to simulate the adrenal cortex in Cushing's disease) with ACTH and three with Metapyralone. In addition these six dogs were given LD_{50} doses of ^{131}I-19-iodocholesterol. Three months after treatment, the adrenal glands of the ACTH-treated dogs were not enlarged, the cortex was thicker than normal, and there were no changes attributable to irradiation. At 3 months, the Metapyralone-treated dogs had enlarged adrenals, widening of the adrenal cortex, and no necrosis or other changes attributable to irradiation. It is concluded that a therapeutic trial of ^{131}I-19-iodocholesterol in the treatment of Cushing's disease is not indicated.

The adrenal glands of dogs (*1*) and humans (*2,3*) have been imaged successfully with a rectilinear scanner and a scintillation camera after administration of ^{131}I-19-iodocholesterol. Previous studies from this laboratory have shown that the uptake of ^{131}I-19-iodocholesterol is increased in patients with adrenal cortical hyperplasia (*4*). The radiation dose to the adrenal glands of the human is calculated to be 30 rads/mCi (*5*). (The radiation dose is 0.94 rads to the total body, 2 rads to the testes, and 2.9 rads to the ovaries.)

These observations encouraged us to evaluate the possible use of ^{131}I-19-iodocholesterol in inhibiting excess steroid production associated with diffuse bilateral adrenocortical hyperplasia associated with ACTH excess.

PRELIMINARY STUDIES

Three normal mongrel female dogs weighing 10–15 kg each were given ^{131}I-19-iodocholesterol intravenously in doses of 27, 44, and 50 mCi, respectively. No adverse effects were noticed. The animals were sacrificed 3 months later. The adrenal glands were grossly and histologically normal (Fig. 1) and the weight of each gland ranged from 455 to 546 mg.

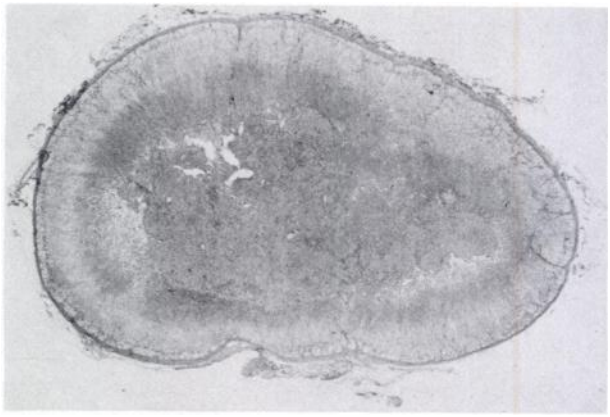

FIG. 1. Normal adrenal gland with separation into medulla (center), zona reticularis (dark area adjacent to medulla), and zonas fasciculata and glomerulosa (lighter areas). (Hematoxylin and eosin, × 5.)

Another three normal mongrel female dogs weighing 10–15 kg each were given ACTH **gel**, 40 units intramuscularly daily for 21 days, and then were given ^{131}I-19-iodocholesterol intravenously in single doses of 40, 41, and 61 mCi, respectively. The dog that received 41 mCi died several hours after the dose was administered. Although the cause of death was unknown, it may have been related to excessive anesthesia. The other two dogs were sacrificed 3 months later. The adrenal glands in both dogs appeared grossly normal but histologically demonstrated changes induced by ACTH stimulation observed in human adrenal glands (Fig. 2).

We were interested in obtaining a suitable model

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FIG. 2. ACTH-stimulated gland with loss of zonation of cortex and conversion of clear cells of zona fasciculata into compact cells. (Hematoxylin and eosin, × 10.)

of adrenal cortical hyperplasia in dogs. Although Cushing's disease occurs in dogs, we were unable to find any animal with spontaneous Cushing's disease that was suitable for a study. Adlin and Channick reported the effects of Metapyralone in normal dogs (*5*). The administration of Metapyralone in doses of 100 mg/kg daily for 2 weeks produced hypertension, hypokalemia, and adrenal cortical hyperplasia. The present experiment was designed to induce adrenal cortical hyperplasia with Metapyralone in dogs and to determine the effects of a large dose of ^{131}I-19-iodocholesterol on the adrenal glands.

MATERIALS AND METHODS

The University of Michigan Nuclear Pharmacy prepared the ^{131}I-19-iodocholesterol as previously described (*6*). The material was formulated as a solution of 10% ethanol, 0.2% polysorbate-80, and 0.9% sodium chloride. The specific activity of the administered ^{131}I-19-iodocholesterol was 500 μCi/mg. A conventional scanner with a 5-in. NaI(Tl) crystal and a 3½-in. fine-focus collimator was used for imaging the adrenal glands. The collimator was placed as near as possible to the backs of the dogs. A scintillation camera (Pho/Gamma III with a 1,000-hole collimator) was used for ^{131}I-19-iodocholesterol imaging and determination of percent uptake using our previously described techniques (*1–4*).

Three female beagles weighing 10–12 kg were maintained on commercial dog food. For adrenal scanning 1 mCi of ^{131}I-19-iodocholesterol was injected intravenously, usually in a volume of less than 1 ml. The dogs were sedated with 1 ml of intramuscular Innovar and anesthetized with 25 mg/kg pentobarbital for administration of the tracer doses and for the scanning procedures. Systolic blood pressures were measured at weekly intervals. Posterior projections of the adrenal glands were obtained with the rectilinear scanner and scintillation camera 6–12 days after injection of the tracer doses.

After the initial scans had been obtained and percent uptakes determined, the dogs were given 100 mg/kg of Metapyralone (mixed with commercial dog food) daily at 10:00 am and 5:00 pm. After 6 weeks to 3 months of Metapyralone treatment, the adrenal scans were repeated. Each dog was then given 50 mCi of ^{131}I-19-iodocholesterol intravenously (with sodium pentobarbital) in a volume of 12–25 ml. Metapyralone was omitted on the day ^{131}I-19-iodocholesterol was given but was continued for 3 months after the therapeutic injection. In two of the dogs adrenal scans and percent uptakes were obtained again 3 months after the large dose of ^{131}I-19-iodocholesterol. The dogs were then sacrificed, necropsies were performed, and radioactivity was assayed in various tissues by methods previously described (*1*).

RESULTS

Although all of the dogs lost 1–3 kg of weight during treatment with Metapyralone, probably due to anorexia, the animals appeared to be in good health. Arterial blood pressure increased during the administration of Metapyralone and remained elevated for the duration of the study in each dog (Table 1).

Radionuclide studies. In the radionuclide images, the adrenal glands were clearly demarcated in all the dogs before and during Metapyralone therapy. The adrenal glands tended to enlarge with Metapyralone administration.

The radioactive uptake of ^{131}I from ^{131}I-19-iodocholesterol increased with Metapyralone-induced ACTH excess bilateral adrenocortical hyperplasia (Table 1).

Pathology. The adrenal glands of the ACTH-treated dogs were not enlarged after 3 months of treatment with ACTH. The cortex was thicker than normal and there were no changes attributable to irradiation. In the dogs treated with Metapyralone the adrenal cortex was similar histopathologically to those treated with ACTH but the adrenals were enlarged.

Several hours after the injection of 50 mCi of ^{131}I-19-iodocholesterol, Dog 803 became listless and died 24–36 hr after receiving the injection. Necropsy showed only hemorrhage in the gastrointestinal tract. Histologic examination of the adrenal glands dem-

TABLE 1. BLOOD PRESSURE, ADRENAL SIZE, AND PERCENT DOSE PER GRAM ADRENAL UPTAKE OF ^{131}I-19-IODOCHOLESTEROL BEFORE AND AFTER TREATMENT

Dog (No.)	Time of measurement	Systolic blood pressure (mmHg)	Adrenal size (cm)	Adrenal weight (gm)	Percent uptake
803	Initial	105	—	—	0.10
	After 6 weeks on Metapyralone*	125	L-2.5 × 1.2 R-2.8 × 1.3	—	0.27
	At sacrifice†	—	—	L-1.4 R-1.3	—
804	Initial	90	L-1.4 × 2.8 R-1.4 × 0.8	—	0.11
	After 6 weeks on Metapyralone	130	L-2.0 × 1.0 R-1.8 × 1.0	—	0.18
	After 3 months on ^{131}I-19-iodocholesterol	130	L-2.6 × 1.3 R-2.4 × 1.3	—	—
	At sacrifice	—	L-2.1 × 1.2 × 0.6 R-2.4 × 1.1 × 0.6	L-0.878 R-0.898	L-0.135 R-0.143
805	Initial	105	—	—	0.19
	After 6 weeks on Metapyralone	140	L-2.5 × 1.0 R-2.8 × 1.0	—	0.45
	After 3 months on ^{131}I-19-iodocholesterol	140	L-2.6 × 1.0 R-2.0 × 1.0	—	0.34
	At sacrifice	—	L-2.5 × 1.0 × 0.7 R-2.6 × 1.0 × 0.5	L-0.950 R-0.810	L-0.136 R-0.161

* Followed by intravenous therapy dose of 50 mCi of ^{131}I-19-iodocholesterol in each dog.
† Death at 24–36 hr after dose.

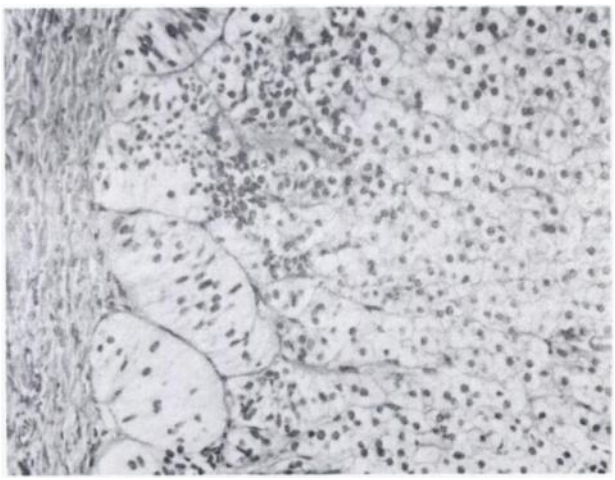

FIG. 3. Normal zona glomerulosa (nodular subcapsular area) and zona fasciculata (small clear cells). (Hematoxylin and eosin, × 260.)

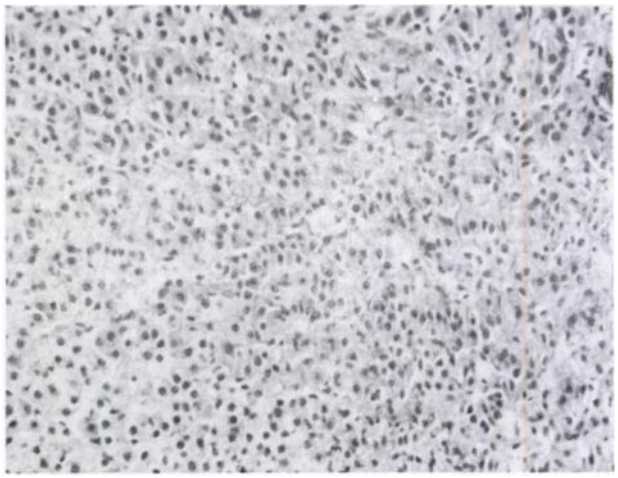

FIG. 4. Normal reticulosis with compact cells. (Hematoxylin and eosin, × 260.)

onstrated marked cortical hyperplasia. The left adrenal gland weighed 1.4 gm and the right adrenal, 1.3 gm. Dogs 804 and 805 had no apparent adverse effects from the injection of 50 mCi of ^{131}I-19-iodocholesterol. Ten to 12 weeks after the therapy dose was given, the adrenal scans were repeated, percent uptake was computed, and then the dogs were sacrificed. Necropsy showed no gross lesions except enlargement of the adrenal glands.

Figures 1, 3, and 4 illustrate adrenal glands taken from one of the dogs that was given a single dose of ^{131}I-19-iodocholesterol without special measures to stimulate the adrenal cortex. The histology of the adrenal cortex is normal. Figure 1 illustrates the two zones of the cortex, a dark area next to the medulla and a light area next to the capsule. In the region immediately beneath the capsule, there are nodules of small clear cells, presumably the zona glomeru-

losa, and beneath that the large clear cells of the zona fasciculata (Fig. 3). Immediately adjacent to the medulla is the area with dark compact cells comparable to the zona reticularis in the human (Fig. 4). There was no necrosis or other change induced by the radioisotope.

ACTH stimulation induced a cortex thicker than normal and alteration of the normal zonation of the cortex, but the glands were not grossly enlarged (Fig. 2). The cells in the zona reticularis and the zona fasciculata are now composed entirely of compact cells. The zona glomerulosa is not as prominent as that found in the normal animal.

In dogs receiving Metapyralone and high doses of radioactive iodocholesterol there was marked widening of the adrenal cortex with loss of the normal zonation and gross enlargement of the gland (Fig. 5). The changes in the cortex were similar to those induced by stimulation with ACTH, i.e., conversion of the clear cells of the zona fasciculata into the compact cells of the zona reticularis (Figs. 6 and 7). No necrosis or other changes attributable to the effect of the radioactive isotope were observed.

Tissue distribution of radioactivity. Tissues were obtained from Dogs 804 and 805 for measurement of radioactivity. The left adrenal gland in Dog 804 contained 0.135% of the dose per gram and the right, 0.143%. In Dog 805 the left adrenal gland contained 0.136% of the dose per gram and the right adrenal, 0.161%. The ovaries from Dog 804 contained 0.009% of the dose per gram and in Dog 805 the ovary contained 0.012%. Other tissues including the liver, skeletal muscle, and kidneys from both dogs contained less than 0.004% of the dose per gram.

FIG. 5. Metapyralone-treated gland with marked cortical hyperplasia. (Hematoxylin and eosin, × 5.)

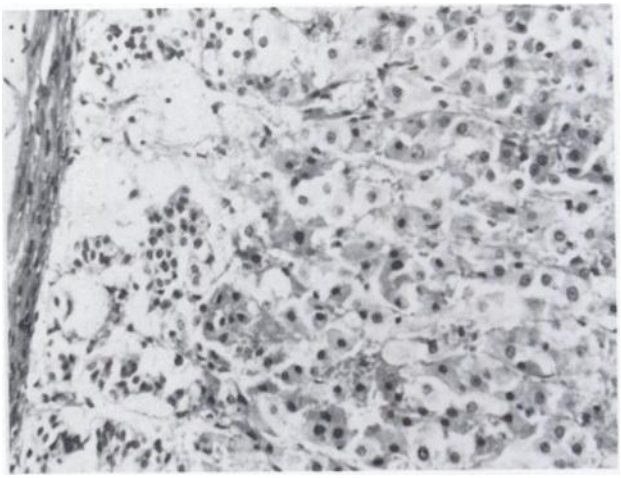

FIG. 6. Metapyralone-treated gland with atrophy of zona glomerulosa and conversion of clear fasciculata cells into compact cells. (Hematoxylin and eosin, × 260.)

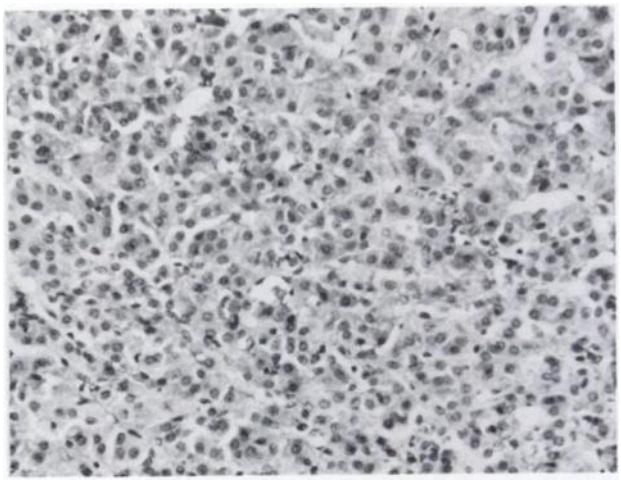

FIG. 7. Metapyralone-treated gland with increase in compact cells in zona reticularis. (Hematoxylin and eosin, × 260.)

DISCUSSION

Adrenal cortical hyperplasia was induced by treatment with Metapyralone in normal dogs. Enlargement of the adrenal glands was demonstrated by radionuclide imaging. There was increased adrenal cortical function reflected by the percent uptake of the administered dose of ^{131}I-19-iodocholesterol. At necropsy, the adrenal glands were larger than those found in similar-sized control animals and showed marked adrenocortical hyperplasia. The persistently elevated blood pressures provided additional evidence that adrenal cortical hyperplasia had been produced and was persisting without functional damage by irradiation from the adrenal content of ^{131}I.

The cause of death in two dogs, in one case after administration of ACTH and in the other after Metapyralone following administration of a large dose of ^{131}I-19-iodocholesterol, is unknown.

The dogs surviving the large doses of ^{131}I-19-

iodocholesterol had no detectable adverse changes in any organs. The adrenal cortical hyperplasia induced by Metapyralone was not altered. There was no necrosis, fibrosis, or other evidence of destructive changes induced by the radioisotope.

Iodine-131-19-iodocholesterol is not effective in destroying a drug-induced adrenal cortical hyperplasia in the dog. It is reasonable to conclude from these data that treatment of bilateral cortical hyperplasia (Cushing's syndrome) with therapeutic doses of ^{131}I-19-iodocholesterol would not be effective.

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